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Supplementary Protocol

QIAseq® FastSelect™ with SMART-Seq® v4 Ultra® Low Input RNA Kit

Most QIAseq FastSelect Kits , may be used with SMART-Seq v4 Ultra Low Input RNA Kit for Sequencing (Takara, cat. nos. 634893, 634892, 634891, 634890, 634889, 634888)

For QIAseq FastSelect –5s/16s/23s Kits, please contact QIAGEN technical support for a modified protocol.

All components of QIAseq FastSelect should be stored at –30°C to –15°C in a constant-temperature freezer.

Further information

- *QIAseq FastSelect Handbook:* www.qiagen.com/HB-2929
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Important points before starting

- The SMART-Seq v4 Ultra Low Input RNA Kit for Sequencing is required for use with this protocol.
- Due to the template switching-based approach of SMARTer, it is important to follow the modified protocol. Otherwise, the library prep will not work.
- Refer to "V. Protocols" section of the *SMART-Seq v4 Ultra Low Input RNA Kit for Sequencing User Manual* (www.takarabio.com/assets/114825).

Procedure

1. Thaw the tube(s) from the QIAseq FastSelect RNA Removal Kit. Mix by vortexing and then briefly centrifuge to collect residual liquid from the sides of the tubes.
2. Make 0.1x FastSelect by combining 1 µL of FastSelect with 9 µL Nuclease-Free Water (scale up as needed). Mix by vortexing and then briefly centrifuge to collect residual liquid from the sides of the tubes.
3. Perform step A.1in "V. Protocols" section of the *SMART-Seq v4 Ultra Low Input RNA Kit for Sequencing User Manual*.
4. Perform step A.2 in section "V. Protocols" of the *SMART-Seq v4 Ultra Low Input RNA Kit for Sequencing User Manual*.
5. Perform step A.3 section "V. Protocols" of the *SMART-Seq v4 Ultra Low Input RNA Kit for Sequencing User Manual* (and follow the footnotes in the user manual), but prepare Table 1 as follows:

Table 1. Sample Preparation Guidelines

Components	Negative Control (µL)	Positive Control (µL)	Test Sample (µL)
10x Reaction Buffer	1	1	1
Nuclease-Free Water	8.5	Up to 7.5	Up to 7.5
Diluted Control RNA*	–	1-8.5	–
Sample	–	–	1-8.5
FastSelect rRNA Removal reagent (diluted to 0.1x)	1	1	1
Total Volume	10.5	10.5	10.5

* (From the *SMART-Seq v4 Ultra Low Input RNA Kit for Sequencing User Manual*) The Control RNA is supplied at a concentration of 1 µg/µL. It should be diluted in nuclease-free water with RNase Inhibitor (1 µL RNase Inhibitor in a final volume of 50 µL of water) to match the concentration of your test sample. Perform serial dilutions on the Control RNA to obtain the appropriate concentration.

6. Place the 10.5 µL samples on ice and add 2 µL of 3′ SMART-Seq CDS Primer II A (12 µM) for a total volume of 12.5 µL. Mix well by gently vortexing and then spin the tube(s) briefly to collect the contents at the bottom of the tube.

Note: If you are performing 17 or more PCR cycles (From the SMART-Seq v4 Ultra Low Input RNA Kit for Sequencing User Manual, refer to Table 2 for PCR cycling guidelines), use 1 µL of the 3′ SMART-Seq CDS Primer II A. Keep the final volume at 12.5 µL by adding additional nuclease-free water or increasing the volume of your sample. Keep the volume of 10X Reaction Buffer at 1 µL regardless of the number of PCR cycles.

7. Perform the following incubation (Table 2) in place of step A.5. Follow the recommendation in the notes though.

Table 2. Combined QIAseq Stranded fragmentation and FastSelect hybridization protocol

Step	Time and temperature (°C)
1	3 min at 72
2	1 min at 70
3	1 min at 65
4	1 min at 60
5	1 min at 55
6	2 min at 37
7	2 min at 25
9	Hold at 4

8. Continue with step A.6 and on from the *SMART-Seq v4 Ultra Low Input RNA Kit for Sequencing User Manual*.

Document Revision History

Date	Changes
10/2024	Initial release.

Scan QR code for the *QIAseq FastSelect Handbook*.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual.